Behavior of Chromatography Columns with a Varying Stationary Phase: I. Segmented Liquid Phase Loadings*

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Abstract

The chromatographic performance of columns comprised of segments with different liquid phase loading is evaluated with respect to efficiencies, resolution, and solute capacity factors. Columns were constructed of segments having 10-5-3-1% and 15-5-3-2% Carbowax 20M coated on 80/100 mesh Chromosorb W and 10-5-3-1% SP2100 on 100/120 mesh Supelcoport. Their behavior as a function of flow direction was studied. Special advantages with respect to separability or analysis time can be selected by a choice of flow direction. Operation of a segmented loaded column in a high-to-low-percent loading configuration may provide increased sample capacity and improved separation whereas the reverse configuration, under the same experimental conditions, offers reduced retention and greater column efficiency.

Introduction

In order to achieve sufficient resolution in analysis of complex samples by gas chromatography (GC), temperature or flow programming has been routinely employed. Programmed-temperature GC provides maximum resolution for the analysis of wide-range boiling mixtures with a minimum of analysis time. Flow programming offers these features as well and can be utilized for separation of thermally sensitive solutes and with liquid phases exhibiting high "bleed" at their maximum operating temperature. Purnell (1) suggested that a GC column in which the capacity factor k ', of the solute varied within the column might exhibit capabilities similar to those offered by programmed-temperature GC.

Locke, Bunting, and Meloan (2,3) obtained excellent agreement between theoretical predictions and experimental results for multi-stage, gradiently loaded squalane columns prepared by linearly or exponentially decreasing the percent loading of squalane from beginning to end of the column. Locke and Meloan (2) observed improved column efficiency and resolution of solutes with low or intermediate k ' on a linearly decreasing gradient column, with k ' being reduced to 50% of its value on a conventional column having a liquid loading corresponding to the initial k ' on the gradient column. Later, Bunting and Meloan (3), utilizing an exponential gradient column, found superior resolution and efficiency with shorter retention times than either conventional, uniformly loaded columns or linear gradient columns of the same average liquid loading.

This study is concerned with the behavior of columns comprised of segments with varying loads of the stationary phase, as a means of decreasing retention and analysis time of solutes that would normally be more strongly retained on a conventional column containing the mean loading of the segmented column. Therefore, this technique has a significant advantage, namely solute elution at a lower column temperature coupled with decreased liquid-phase bleeding. Karger and Cooke discussed the role of the lightly loaded columns, uniformly coated, which yield decreased retention (4). This condition is highly desirable in quantitative and GC/MS analyses. In the present investigation a segmented column, 8 ft in length, was composed of 2-ft sections containing 15- or 10-5-3- and 2% Carbowax 20M coated on inert solid support. A 10-ft column, consisting of 2.5-ft sections of 10-5-3- and 1% SP2100 on Supelcoport, was also prepared.

A packed GC column has an inherent linear velocity gradient that is a consequence of the pressure drop across the column, with carrier flow rate being greatest at the outlet and least at the column inlet. Therefore, depending on the operational configuration of a segmented column, namely, high-to-low-percent loading *vs.* the reverse arrangement (i.e., 2-3-5-10%), unique pairs of fundamental chromatographic parameters should be observed. This uniqueness is associated with the position of the highest loaded segment in the flow profile. Some practical advantages of these columns are described.

Preparation of columns

The segment-loaded columns were prepared from 15-10-5-3- and 2% Carbowax 20M coated on 80/100 mesh Chromosorb W (Applied Science Laboratories and Supelco). Each of the packings was placed with vertical tapping and suction into a

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2-ft length of 1/8-in o.d. stainless steel tubing previously rinsed with methylene chloride and dried in a stream of nitrogen. Four sections were then connected with 1/8-in o.d. Swagelok LDV unions to yield an 8-ft length of a 15-5-3-2% or a 10-5-3-2% segment-loaded Carbowax 20M column. A ten-foot, segment-loaded column comprised of 2.5-ft segments of 10-5-3- and 1% SP2100 on 100/120 mesh Supelcoport was prepared in a similar fashion. The columns were conditioned overnight at 175°C with a nitrogen flow of 30 mL/min. The highest loaded end was connected to the injection block during the conditioning process. The main consideration for the selection of the stationary phases was the availability of commercially prepared packings in the authors' laboratories, the intention being to study their representative behavior.

Equipment

Most of the chromatograms were obtained on a Bendix model 2200 GC, and some were replicated on a Perkin-Elmer model 3920 GC. Flow rates of hydrogen and air for the FID detectors were carefully maintained at 30 and 200 ml/min, respectively. Nitrogen was used as carrier gas. The chromatographic peaks were displayed on a Hewlett-Packard model 7128A strip chart recorder with a range of 1 mv and a chart speed of 1 in/min. Sample solutions containing 5% (v/v) of each component were prepared and injected at the indicated volume with a Hamilton 10-μl syringe. All chromatograms were generated at a detector range control of 100 and an attenuation suitable for all peaks to remain on scale.

Results and Discussion

In Figure 1 separations of a seven-component aromatic mixture are illustrated on A) an 8-ft 5% Carbowax 20M column, B) an 8-ft 10-5-3-2% Carbowax 20M column (10% end attached to injection block), and C) 2-3-5-10% Carbowax 20M column (same column as in B, but with the 2% loaded end as inlet). Column temperature and flow rate were maintained at 100°C and 30 ml/min for these separations. Inspection of these chromatograms shows the enhanced resolution of the segmented columns compared to that of the uniformly loaded 5% Carbowax 20M column corresponding to the mean loading of the segmented columns. In addition, comparison of B and C qualitatively reflects the improved separation obtained with a high-to-low-percent loading, while the very same column operated in the reversed configuration provides a substantial reduction in analysis time. Since all experimental parameters—such as column temperature and inlet pressure, flow rate and size of injected sample vapors—were held constant, the conclusion is that the direction of the flow plays an important role in the determination of resolution and analysis time.

To verify the uniqueness of these separations, when the same set of chromatographic parameters were used, the retention time of the last eluting component of the mixture, bromobenzene, on the 10-5-3-2% column was found to correspond to that obtained with a 4-ft length of 10% loaded Carbowax 20M column. Similarly, a 3-ft segment of 10% Carbowax 20M yielded the same retention of this solute as the 2-3-5-10% column.

If additional resolution is desired, a more highly loaded segment can be incorporated into the column. A 2-ft section of 15% Carbowax 20M on 80/100 mesh Chromosorb W was substituted for the 10% segment and the column reconditioned at 175°C for 24 hr. The performance of this column in each flow direction is illustrated in Figures 2 and 3 for the separation of aromatics and alcohols. Again, improved separation is

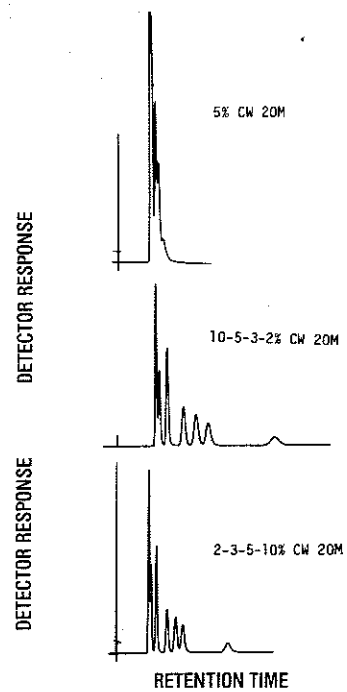

Figure 1. Separation of aromatic mixture on various CW20M columns: conditions: column temperature, 100°C, flow rate, 30 ml/min; injection temperature, 225°C; detector temperature, 250°C; chart speed, 1 in/min; sample size, 1.0 μl sample vapor; sample components in order of elution: benzene, fluorobenzene, toluene, ethylbenzene, cumene, chlorobenzene, and bromobenzene

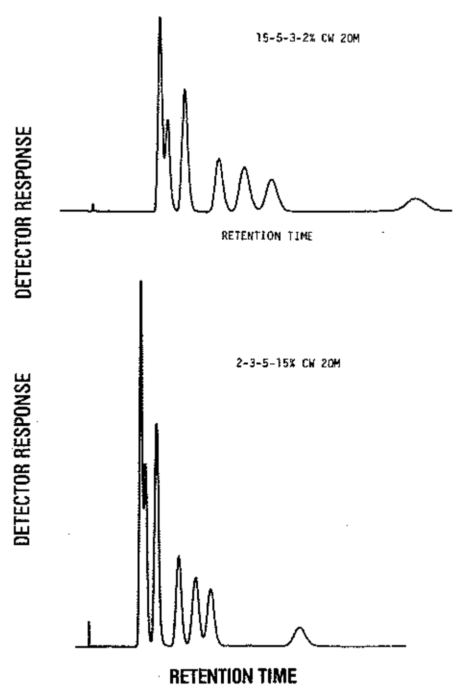

Figure 2. Separation of aromatic mixture on 15-5-3-2% and 2-3-4-15% CW20M columns: conditions same as Figure 1

associated with the high-to-low configuration whereas reduced analysis time is seen for the low to high, illustrated in Figure 4 for the separation of several aromatic hydrocarbons.

Retention data are tabulated in Tables I and II for various solutes on the two systems considered. For all solutes studied, reduced retention is again observed in the low-to-high direction. All operating parameters, such as inlet pressure, sample size, flow rate, injector, column and detector temperatures, were carefully controlled during acquisition of these data.

In Table III, fundamental chromatographic parameters for several *n*-alkanes are listed. The efficiency of SP2100 column was measured by the computation of the number of theoretical plates, N, from the expression:

$$N = 5.55\ (t_r/W_h)^2 \qquad \text{Eq. 1}$$

where W_h is the width of the chromatographic peak at half-height. However, a more accurate measure of separation power is the determination of effective plate number, N_{eff}, from the equation:

$$N_{eff} = 5.55\ (t'_r/W_h)^2 \qquad \text{Eq. 2}$$

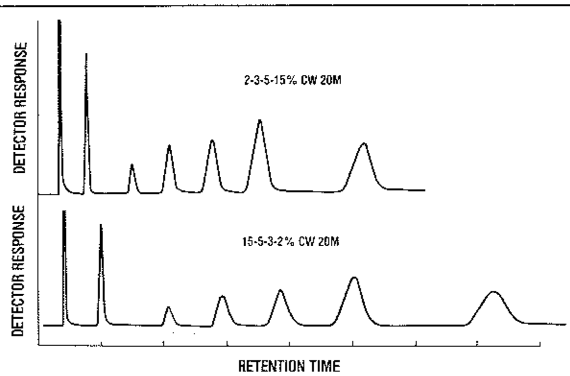

Figure 3. Separation of alcohol mixture on 15-5-3-2% and 2-3-5-15% CW20M columns: Column temperature, 85°C; other conditions are the same as in Figure 1. Sample components in order of elution: methanol (solvent), isopropanol, 3-methyl-3-pentanol, 1-pentanol, 2-ethyl-1-butanol, 1-hexanol, and cyclohexanol

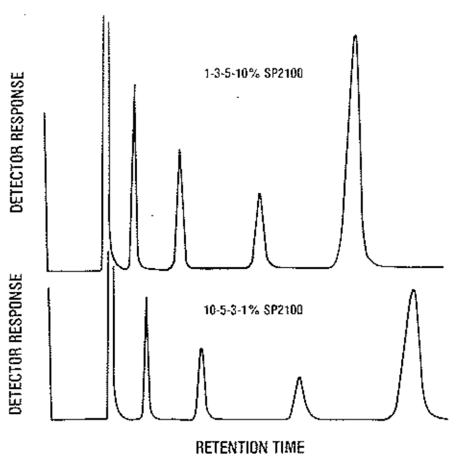

Figure 4. Separation of aromatic on 10-5-3-1% and 1-3-5-10% SP2100 columns: Column temperature, 90°C; flow rate, 20 ml/min; other conditions are the same as in Figure 1. Sample components in order of elution: CH_2Cl_2 (solvent), benzene, toluene, ethylbenzene, and cumene

where t'_r is the adjusted retention time. Column efficiency can alternatively be measured by the separation number, TZ, where:

$$TZ = \frac{t_{r_2} - t_{r_1}}{W_{h_1} + W_{h_2}} - 1 \qquad \text{Eq. 3}$$

Examination of the W_h, N, N_{eff}, and TZ data in Table III shows that reduced retention is accompanied by improved col-

Table I. Retention Data on Segmented Loaded Carbowax 20M Column*

Solute	t'_r (sec) 15-5-3-2% Configuration	t'_r (sec) 2-3-5-15% Configuration
Column Temperature: 90°C		
Methanol	25	17
Ethanol	68	45
1-propanol	135	92
1-butanol	261	178
1-pentanol	908	624
Benzene	45	32
Toluene	86	59
Ethylbenzene	146	99
Cumene	197	132
Column Temperature: 140°C		
Undecane	28	18
Dodecane	47	32
Tridecane	76	52
Tetradecane	121	85
Pentadecane	192	137
Hexadecane	305	220
Heptadecane	486	354
Octadecane	776	572
Nonadecane	1887	1430

*Injection temp: 225°C; detector temp: 240°C; flow rate: 20 ml/min; average of triplicate determinations

Table II. Retention Data on Segmented Loaded SP2100 Column*

Solute	t'_r (sec) 10-5-3-1% Configuration	t'_r (sec) 1-3-5-10% Configuration
n-Pentane	15	14
n-Hexane	33	22
n-Heptane	73	60
n-Octane	151	123
n-Nonane	306	246
1-Hexene	30	25
1-Heptene	76	59
1-Octene	164	129
Benzene	54	44
Toluene	113	93
Ethylbenzene	231	179
Cumene	353	282
1-Propanol	22	17
1-Butanol	51	41
1-Pentanol	111	88
1-Heptanol	470	380

*Column temp: 90°C; injector and detector temp: 225°C; flow rate: 20 ml/min; average of triplicate determinations

Table III. Fundamental Chromatographic Parameters of SP2100 Segmented Column* for *n*-Alkanes

Solute	t_r (sec)		t_r' (sec)		W_h (sec)		k'		N		N_{eff}		TZ	
	a**	b***	a	b	a	b	a	b	a	b	a	b	a	b
Undecane	87	76	39	28	3.3	2.4	0.79	0.59	3813	5314	746	725	1.38	1.50
Dodecane	105	89	57	41	4.1	2.9	1.16	0.86	3550	5202	1022	1120	1.69	1.90
Tridecane	130	109	82	60	5.3	3.5	1.68	1.26	3318	5286	1306	1635	2.11	2.44
Tetradecane	168	137	120	89	6.8	4.7	2.46	1.84	3332	4712	1687	1979	2.32	2.59
Pentadecane	221	176	173	128	9.3	6.2	3.57	2.65	3129	4097	1910	2160	2.42	2.90
Hexadecane	298	235	250	187	13.1	8.8	5.16	3.88	2923	3941	2050	2492	2.54	3.02
Heptadecane	411	321	363	273	19.0	12.4	7.50	5.65	2623	3734	2091	2696		

*Column temp: 200°C; injector: 225°C; detector: 240°C; flow rate: 20 ml/min; average of triplicate determinations
**(10-5-2-1%) SP2100 configuration
***(1-3-5-10%) SP2100 configuration

umn efficiency in the low-to-high mode. For example, the k' of *n*-heptadecane in the 1-3-5-10% configuration (k' = 5.65) is diminished by 25% with a corresponding 30% gain in N_{eff}. The observation of this rather unique chromatographic behavior suggests further study of the performance of segmented columns, which may lead to a number of practical advantages. Moreover, the results of this investigation further demonstrate that operation in a high-to-low direction provides increased capacity, which is important in preparative and process chromatography; whereas the same column operated with flow reversal exhibits enhanced column efficiency and reduced time of analysis, which are significant considerations in analytical separations. Therefore, the authors now suggest that a single GLC column, gradiently loaded, can serve both as an analytical and a semi-preparative column.

With respect to the existence of pressure drop previously mentioned, the volumetric flow rate, V, at any point, X, in a GC column of length, L, can be calculated from the following expression:

$$\frac{X}{L} = \frac{(P_i/P_o)^2 - (V_o/V)^2}{(P_i/P_o)^2 - 1} \qquad \text{Eq. 4}$$

where P_i/P_o is the inlet-to-outlet pressure ratio and V_o is outlet flow rate. Typical profiles of the velocity change or gradient along the length of a GC column are presented for several values of P_i/P_o in Figure 5, which illustrates that volume flow rate is greater at the column outlet than at the inlet and is the resultant of the pressure drop across the column. Thus, when the high-load end of a gradient column is attached to the injection block, the low flow rate at the column inlet, together with the high loading (10 or 15 % in this study), produces greater band broadening, longer retention times, and lower column efficiency than in the reverse direction. In the latter case, at identical chromatographic conditions with the highest loaded segment attached to the detector, the flow rate rapidly increases toward the column outlet and overcomes the retarding influence of the high-percent loading, yielding reduced retention of all solutes and greater column efficiency. A theoretical treatment, based on classical GLC partition theory, appears in the following section and describes the column behavior reported in this paper.

In Figure 6, HETP *vs.* flow rate curves are presented for the columns under consideration. Although a gradient column operated in either configuration exhibits the same optimum flow rate associated with the minimum HETP, the overall greater efficiency of a column operated in the low-to-high load configuration is displayed by the lower curve for each column.

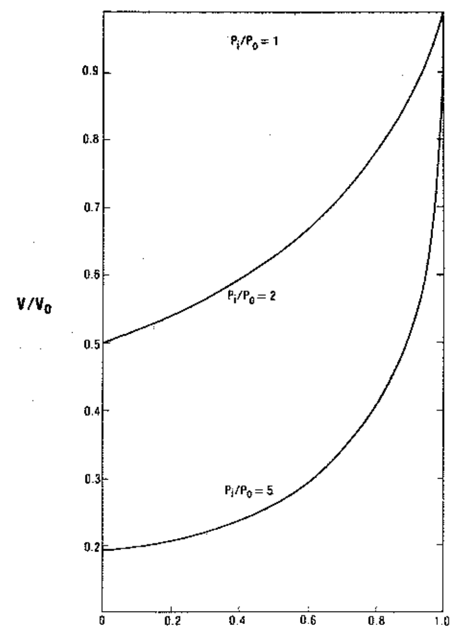

Figure 5. Plot of V/V_o versus X/L with P_i/P_o as a parameter

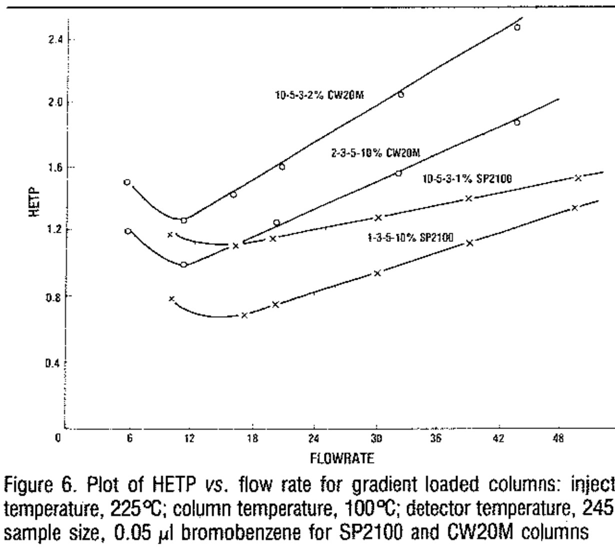

Figure 6. Plot of HETP *vs.* flow rate for gradient loaded columns: injection temperature, 225°C; column temperature, 100°C; detector temperature, 245°C; sample size, 0.05 µl bromobenzene for SP2100 and CW20M columns

Currently the performance of other segmented columns is being studied in the authors' laboratories from a theoretical viewpoint, with emphasis on the role of P_i/P_o and the optimization of percent loadings comprising the gradient. Moreover, the practical role of gradient columns in the GC of thermally sensitive solutes, and the reduction in column bleeding provided by a decreasing gradient loading, important in GC/MS analyses, are being investigated. Also, the effects of the conditioning temperature and prolonged operation at elevated temperatures on the migration of stationary phases in these columns are being studied.

Extension of this approach to gas-solid chromatography has shown enhanced column efficiencies, improved resolution, and reduced retention of various solutes when a segmented column was constructed with porous silica beads of decreasing specific surface area and compared to a uniformly loaded porous silica bead column of the equivalent surface area. These results will be reported in a subsequent paper.

The principles outlined here have been applied to LC. The results of studies in both the liquid-solid and reverse-phase modes of HPLC will also be reported in this series of papers.

Theoretical Considerations

The flow dynamics associated with a packed chromatographic column can be conveniently described with the one-dimensional Fick equation:

$$D\frac{\delta^2 C}{\delta x^2} = v\frac{\delta C}{\delta x} + \frac{\delta C}{\delta t} + \frac{1}{\beta}\frac{\delta n}{\delta t} \qquad \text{Eq. 5}$$

where C and n are, respectively, the concentrations of the analyte in the mobile and stationary phases, β is the fractional void volume in the bed, D is the diffusion coefficient of the analyte molecules in the mobile phase, v is the velocity of the mobile phase through interstices of the bed, and x and t are the distance and time, respectively. With the assumptions that v and β are constants, Lapidus and Amundson have derived the general solutions of Equation 5 for the cases of linear adsorption and absorption equilibrium isotherms (5). These solutions constitute the basis of the famous Van Deemter equation.

In the case of segmented loaded GC, both v and β are x-dependent parameters. Equation 5 is usually not solved, and a more restricted assumption is needed if explicit solutions are required. This can be postulated from the fact that longitudinal diffusion only causes band broadening, not retention shifting. Under normal chromatographic conditions the band broadening due to diffusion is relatively small as compared to other sources. Therefore, it is conceivable to neglect the second order derivative term in Equation 5. In addition, if instantaneous equilibrium,

$$\frac{\delta n}{\delta t} = K\frac{\delta C}{\delta t} \qquad \text{Eq. 6}$$

is assumed to be established at each point of the column during elution, Equation 5 can be further simplified to yield the following expression:

$$\frac{\delta C}{\delta x} = -\frac{1+k'}{v}\frac{\delta C}{\delta t} \qquad \text{Eq. 7}$$

where $k = K/\beta$ is commonly referred to as the capacity factor. The solution of Equation 7 gives a Gaussian concentration profile with the maximum located at the retention time, t_R:

$$t_R = \int_O^L \frac{1+k'}{v}dx. \qquad \text{Eq. 8}$$

When the column of length, L, is segmented, loaded with liquid phase, and operated either in decreasing or increasing order, then the retention time can be expressed as follows:

$$t_R = \int_O^{\ell} \frac{(1+k'_1)\,dx}{v} + \int_{\ell}^{2\ell} \frac{(1+k'_2)\,dx}{v} + \int_{2\ell}^{3\ell} + \frac{(1+k'_3)\,dx}{v}$$

or

$$t_R = \sum_{i=1}^{n} (1+k'_i) \int_{\ell_{i-1}}^{\ell_i} \frac{dx}{v} \qquad \text{Eq. 9}$$

where k'_i is the capacity factor of the solute and L_i is the column length measured from the column inlet to the end of the *i*th segment. Because of the compressibility of the carrier gas, the linear velocity, v, at a point, x, can be represented as:

$$\frac{1}{v} = \frac{1}{v_o}\sqrt{\left(\frac{P_i}{P_o}\right)^2 - \left\{\left(\frac{P_i}{P_o}\right)^2 - 1\right\}\frac{x}{L}} = \frac{1}{v_o}\sqrt{a-bx} \qquad \text{Eq. 10}$$

where $a = (P_i/P_o)^2$; $b = [(P_i/P_o)^2 - 1]/L$; P_i/P_o is the inlet-to-outlet pressure ratio, and v_o is the outlet linear velocity. The integration in Equation 5 can be subsequently carried out to give:

$$t_R = \sum_{i=1}^{n} \frac{2}{3}\frac{(1+k'_i)}{bv_o}\left[(a-b\ell_{i-1})^{3/2} - (a-b\ell_i)^{3/2}\right] \qquad \text{Eq. 11}$$

If all segments are of the same length, i.e., $L = L/n$, then Equation 7 becomes:

$$t_R = \sum_{i=1}^{n} \frac{2(1+k'_i)}{3bv_o}\left\{[a-(i-1)b\ell]^{3/2} - (a-ib\ell)^{3/2}\right\} \qquad \text{Eq. 12}$$

Inspection of Equations 7 and 8 indicates that t_R is dependent on the arrangement of the segmented loadings; the resulting order of k ′ values produces a genuine influence on retention times when flow direction is changed.

Finally, in the trivial case where the column is uniformly loaded, i.e., $k'_i = k'$:

$$t_R = \frac{2(1+k')}{3bv_o}[a^{3/2} - 1] = \frac{2L(1+k')}{3v_o}\frac{(P_i/P_o)^3 - 1}{(P_i/P_o)^2 - 1} \qquad \text{Eq. 13}$$

$$t_R = \frac{L}{j v_o}(1+k') = \frac{L}{\bar{v}}(1+k') = t_o(1+k') \qquad \text{Eq. 14}$$

where j and $\bar{v}$ are the James-Martin compressibility factor and average linear carrier gas velocity, respectively. The significance of Equation 10 in GC is well established (6).

The underlying features of Equations 6, 7, and 8 predict that a segmented, loaded GC column operated in high-to-low-percent loading configuration (increasing phase ratio, β, of segments) should offer higher capacity and separability, whereas the reverse configuration (decreasing phase ratio of segments) provides reduced retentions. These observations are derived from consideration of the flow profile in a packed GC column, in which the linear velocity is not constant but varies throughout the length of the column. The arrangement of the loadings in the flow profile should provide unique pairs of solute chromatographic parameters as a function of flow direction.

The authors have employed the principles previously outlined in a computer-simulation study of segmented, loaded column performance. The various column and solute properties selected are presented in Table IV. Briefly, the behavior of a column 100 cm in length, consisting of four segments that have phase ratios of 10, 1.0, 0.5, and 0.1, was evaluated for an eight-component mixture. Moreover, in the computations, HETP values were generated in accordance with the classical form of the van Deemter equation (1). For simplicity, the contribution of the A and B terms was neglected, since a high-outlet linear velocity was selected. The film thickness, d_f, was calculated from the particle diameter, d_p, by the following expression derived by Purnell (7):

$$d_f = d_p^2 \frac{(k')^2}{16\ K^2} \qquad \text{Eq. 15}$$

Therefore, H values computed for a given segment with linear velocity, v, were then added to obtain the total HETP for a solute.

In Table V, computer-generated retention and efficiency data are presented to verify the experimental observations associated with a variable phase ratio column. Simulated chromatograms for an eight-component mixture at several hypothetical inlet-to-outlet pressure ratios were also generated, and appear in Figure 7.

Table IV. Simulated Chromatographic Conditions for Segmented Loaded Column

Column Parameters	
Inlet Pressure, P_i:	2 atmospheres
Outlet Pressure, P_o:	1 atmosphere
Total Column Length, L:	100 cm
Number of Segments:	4 (each 25 cm in length)
Phase Ratio of Segments, β:	10.0; 1.0; 0.5; 0.1
Outlet Linear Velocity, v_o:	3 cm/sec
Particle Size of Solid Support, d_p:	0.005 cm
Solute Properties	
Diffusion Coefficient of Solute in Stationary Phase, D_S:	1×10^{-5} cm²/sec
Number of Solutes:	8
Distribution Coefficients of Solutes, K:	0.1, 0.5, 1.0, 2.0, 5.0, 7.0, 10.0, 15.0

Table V. Simulated Retention and Efficiency Data on a Segmented Loaded Column

	High to Low Loading		Low to High Loading	
Solute	t_r (sec)	N	t_r (sec)	N
1	72	29	65	40
2	152	25	120	27
3	252	34	189	39
4	452	51	327	64
5	1052	87	739	95
6	1451	105	1013	110
7	2051	125	1426	140
8	3051	148	2116	178

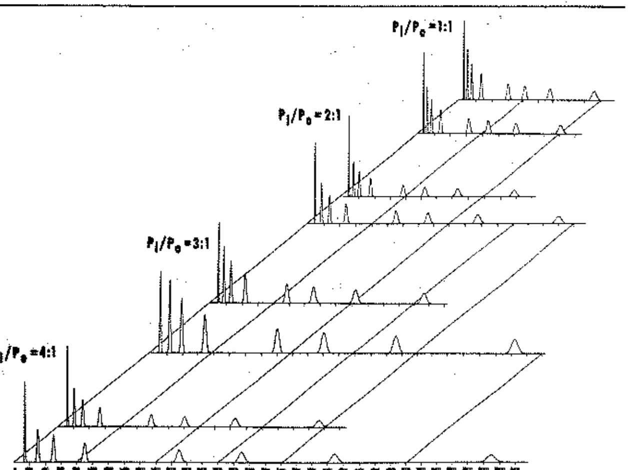

Figure 7. Computer-simulated chromatograms generated from Equations 8 and 11 for the hypothetical values in Table IV: For each pair of chromatograms corresponding to fixed ratios of P_i/P_o, the chromatogram for k′ increasing is shown above that for k′ decreasing.

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